

We claim:

1. A process for preparing ketocarotenoids by cultivating genetically modified organisms which, compared with the wild type, have a modified ketolase activity, and the modified ketolase activity is caused by a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2.
2. A process as claimed in claim 1, wherein organisms which, as wild type, already have a ketolase activity, and the genetic modification brings about an increase in the ketolase activity compared with the wild type, are used.
3. A process as claimed in claim 1, wherein the ketolase activity is increased by increasing the gene expression of a nucleic acid encoding a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2, compared with the wild type.
4. A process as claimed in claim 3, wherein the gene expression is increased by introducing nucleic acids which encode ketolases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2, compared with the wild type, into the organism.
5. A process as claimed in claim 1, wherein organisms which, as wild type, have no ketolase activity are used, and the genetic modification causes a ketolase activity compared with the wild type.
6. A process as claimed in claim 5, wherein genetically modified organisms which transgenically express a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2, are used.
7. A process as claimed in claim 5 or 6, wherein the gene expression is caused by introducing nucleic acids which encode ketolases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the

sequence SEQ. ID. NO. 2, into the organism

8. A process as claimed in claim 5 or 7, wherein nucleic acids comprising the sequence SEQ. ID. NO. 1 are introduced.

5

9. A process as claimed in any of claims 1 to 8, wherein the organisms additionally have an increased activity, compared with the wild type, of at least one of the activities selected from the group of hydroxylase activity and β -cyclase activity.

- 10 10. A process as claimed in claim 9, wherein the gene expression of at least one nucleic acid selected from the group of nucleic acids encoding a hydroxylase, and nucleic acids encoding a β -cyclase, is increased compared with the wild type for the additional increase in at least one of the activities.

- 15 11. A process as claimed in claim 10, wherein the gene expression is increased by introducing at least one nucleic acid selected from the group of nucleic acids encoding a hydroxylase and nucleic acids encoding a β -cyclase into the organism.

- 20 12. A process as claimed in claim 11, wherein nucleic acids which encode a hydroxylase comprising the amino acid sequence SEQ ID NO: 16 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 16 are introduced as nucleic acid encoding a hydroxylase.

- 25 13. A process as claimed in claim 12, wherein nucleic acids comprising the sequence SEQ ID NO: 15 are introduced.

- 30 14. A process as claimed in claim 11, wherein nucleic acids which encode a β -cyclase comprising the amino acid sequence SEQ ID NO: 18 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 20% at the amino acid level with the sequence SEQ ID NO: 18 are introduced as nucleic acid encoding a β -cyclase.

- 35 15. A process as claimed in claim 14, wherein nucleic acids comprising the sequence SEQ ID NO: 17 are introduced.

16. A process as claimed in any of claims 1 to 15, wherein the genetically modified organisms are harvested after cultivation, and subsequently the ketocarotenoids are isolated from the organisms.

17. A process as claimed in any of claims 1 to 16, wherein an organism which is able as starting organism naturally or through genetic complementation or reregulation of metabolic pathways to produce carotenoids is used as organism.
- 5
18. A process as claimed in any of claims 1 to 17, wherein microorganisms or plants are used as organisms.
19. A process as claimed in claim 18, wherein bacteria, yeasts, algae or fungi are used as
- 10 microorganisms.
20. A process as claimed in claim 19, wherein the microorganisms are selected from the group of *Escherichia*, *Erwinia*, *Agrobacterium*, *Flavobacterium*, *Alcaligenes*, *Paracoccus*, *Nostoc*, cyanobacteria of the genus *Synechocystis*, *Candida*, *Saccharomyces*, *Hansenula*, *Phaffia*,
- 15 *Pichia*, *Aspergillus*, *Trichoderma*, *Ashbya*, *Neurospora*, *Blakeslea*, *Phycomyces*, *Fusarium*, *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or *Dunaliella*.
21. A process as claimed in claim 18, wherein plants are used as organism.
- 20 22. A process as claimed in claim 21, wherein a plant selected from the families Ranunculaceae, Berberidaceae, Papaveraceae, Cannabaceae, Rosaceae, Fabaceae, Linaceae, Vitaceae, Brassicaceae, Cucurbitaceae, Primulaceae, Caryophyllaceae, Amaranthaceae, Gentianaceae, Geraniaceae, Caprifoliaceae, Oleaceae, Tropaeolaceae, Solanaceae, Scrophulariaceae, Asteraceae, Liliaceae, Amaryllidaceae, Poaceae,
- 25 Orchidaceae, Malvaceae, Iliaceae or Lamiaceae is used as plant.
23. A process as claimed in claim 22, wherein a plant selected from the plant genera Marigold, Tagetes erecta, Tagetes patula, Acacia, Aconitum, Adonis, Arnica, Aquilegia, Aster, Astragalus, Bignonia, Calendula, Caltha, Campanula, Canna, Centaurea, Cheiranthus,
- 30 Chrysanthemum, Citrus, Crepis, Crocus, Curcubita, Cytisus, Delonia, Delphinium, Dianthus, Dimorphotheca, Doronicum, Eschscholtzia, Forsythia, Fremontia, Gazania, Gelsemium, Genista, Gentiana, Geranium, Gerbera, Geum, Grevillea, Helenium, Helianthus, Hepatica, Heracleum, Hibiscus, Heliopsis, Hypericum, Hypochoeris, Impatiens, Iris, Jacaranda, Kerria, Laburnum, Lathyrus, Leontodon, Lilium, Linum, Lotus, Lycopersicon,
- 35 Lysimachia, Maratia, Medicago, Mimulus, Narcissus, Oenothera, Osmanthus, Petunia, Photinia, Physalis, Phyteuma, Potentilla, Pyracantha, Ranunculus, Rhododendron, Rosa, Rudbeckia, Senecio, Silene, Silphium, Sinapsis, Sorbus, Spartium, Tecoma, Torenia, Tragopogon, Trollius, Tropaeolum, Tulipa, Tussilago, Ulex, Viola or Zinnia is used as plant.

24. A process as claimed in any of claims 1 to 23, wherein the ketocarotenoids are selected from the group of astaxanthin, canthaxanthin, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, adonirubin and adonixanthin.

5 25. A genetically modified organism where the genetic modification

A, in the case where the wild-type organism already has a ketolase activity, increases the activity of a ketolase compared with the wild type and

10 B, in the case where the wild-type organism has no ketolase activity, causes the activity of a ketolase compared with the wild type,

and the ketolase activity which has been increased as in A or caused as in B is caused by a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is
15 derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2.

26. A genetically modified organism as claimed in claim 25, wherein the increasing or causing of the ketolase activity is brought about by an increasing or causing of the gene expression
20 of a nucleic acid encoding a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2, compared with the wild type.

25 27. A genetically modified organism as claimed in claim 26, wherein to increase or cause the gene expression nucleic acids which encode ketolases comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2, are introduced into the organism.

30 28. A genetically modified organism comprising at least one transgenic nucleic acid encoding a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2.

35 29. A genetically modified organism comprising at least two endogenous nucleic acids encoding a ketolase comprising the amino acid sequence SEQ. ID. NO. 2 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 42% at the amino acid level with the sequence SEQ. ID. NO. 2.

30. A genetically modified organism as claimed in any of claims 25 to 29, wherein the genetic modification additionally increases at least one of the activities selected from the group of hydroxylase activity and β -cyclase activity, compared with the wild type.
- 5 31. A genetically modified organism as claimed in any of claims 25 to 30, which is able as starting organism naturally or through genetic complementation to produce carotenoids.
- 10 32. A genetically modified organism as claimed in any of claims 25 to 31, selected from the group of microorganisms or plants.
33. A genetically modified organism as claimed in claim 32, wherein the microorganisms are selected from the group of bacteria, yeasts, algae or fungi.
- 15 34. A genetically modified microorganism as claimed in claim 33, wherein the microorganisms are selected from the group of *Escherichia*, *Erwinia*, *Agrobacterium*, *Flavobacterium*, *Alcaligenes*, *Paracoccus*, *Nostoc*, cyanobacteria of the genus *Synechocystis*, *Candida*, *Saccharomyces*, *Hansenula*, *Pichia*, *Aspergillus*, *Trichoderma*, *Ashbya*, *Neurospora*, *Blakeslea*, *Phycomyces*, *Fusarium*, *Haematococcus*, *Phaedactylum tricornatum*, *Volvox* or
- 20 *Dunaliella*.
35. A genetically modified plant as claimed in claim 32, wherein the plants are selected from the families Ranunculaceae, Berberidaceae, Papaveraceae, Cannabaceae, Rosaceae, Fabaceae, Linaceae, Vitaceae, Brassicaceae, Cucurbitaceae, Primulaceae, Caryophyllaceae,
- 25 Amaranthaceae, Gentianaceae, Geraniaceae, Caprifoliaceae, Oleaceae, Tropaeolaceae, Solanaceae, Scrophulariaceae, Asteraceae, Liliaceae, Amaryllidaceae, Poaceae, Orchidaceae, Malvaceae, Iliaceae or Lamiaceae.
- 30 36. A genetically modified plant as claimed in claim 35, wherein the plants are selected from the plant genera Marigold, *Tagetes erecta*, *Tagetes patula*, *Acacia*, *Aconitum*, *Adonis*, *Arnica*, *Aquilegia*, *Aster*, *Astragalus*, *Bignonia*, *Calendula*, *Caltha*, *Campanula*, *Canna*, *Centaurea*, *Cheiranthus*, *Chrysanthemum*, *Citrus*, *Crepis*, *Crocus*, *Curcubita*, *Cytisus*, *Delonia*, *Delphinium*, *Dianthus*, *Dimorphotheca*, *Doronicum*, *Eschscholtzia*, *Forsythia*, *Fremontia*, *Gazania*, *Gelsemium*, *Genista*, *Gentiana*, *Geranium*, *Gerbera*, *Geum*, *Grevillea*, *Helenium*,
- 35 *Helianthus*, *Hepatica*, *Heracleum*, *Hibiscus*, *Heliopsis*, *Hypericum*, *Hypochoeris*, *Impatiens*, *Iris*, *Jacaranda*, *Kerria*, *Laburnum*, *Lathyrus*, *Leontodon*, *Lilium*, *Linum*, *Lotus*, *Lycopersicon*, *Lysimachia*, *Maratia*, *Medicago*, *Mimulus*, *Narcissus*, *Oenothera*, *Osmanthus*, *Petunia*, *Photinia*, *Physalis*, *Phyteuma*, *Potentilla*, *Pyracantha*, *Ranunculus*, *Rhododendron*, *Rosa*, *Rudbeckia*, *Senecio*, *Silene*, *Silphium*, *Sinapsis*, *Sorbus*, *Spartium*, *Tecoma*, *Torenia*,

Tragopogon, Trollius, Tropaeolum, Tulipa, Tussilago, Ulex, Viola or Zinnia.

37. The use of the genetically modified organisms as claimed in any of claims 25 to 36 as animal or human food.
- 5
38. The use of the genetically modified organisms as claimed in any of claims 25 to 36 for producing ketocarotenoid-containing extracts or for producing animal and human food supplements.
- 10
39. A ketolase comprising the amino acid sequence SEQ. ID. NO. 8 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 8, with the proviso that the amino acid sequence SEQ. ID NO. 4 is not present.
- 15
40. A ketolase comprising the amino acid sequence SEQ. ID. NO. 6 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 6.
- 20
41. A ketolase comprising the amino acid sequence SEQ ID NO. 12 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 70% at the amino acid level with the sequence SEQ ID NO. 12, with the proviso that the amino acid sequence SEQ ID NO. 6 is not present.
- 25
42. A ketolase comprising the amino acid sequence SEQ ID NO. 49 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 50% at the amino acid level with the sequence SEQ ID NO. 49, with the proviso that the amino acid sequence SEQ ID NO. 47 is not present.
- 30
43. A nucleic acid encoding a protein as claimed in any of claims 39 to 42, with the proviso that the sequence SEQ ID NO: 5 is not present.
- 35
44. The use of a protein comprising the amino acid sequence SEQ. ID. NO. 4 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 70% at the amino acid level with the sequence SEQ. ID. NO. 4, and has the property of a ketolase, as ketolase.
45. The use of a protein comprising the amino acid sequence SEQ. ID. NO. 6 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 65% at the amino acid level with the sequence SEQ. ID.

NO. 6, and has the property of a ketolase, as ketolase.

46. The use of a protein comprising the amino acid sequence SEQ ID NO. 47 or a sequence which is derived from this sequence by substitution, insertion or deletion of amino acids and which has an identity of at least 50% at the amino acid level with the sequence SEQ ID NO. 47 and which has the property of a ketolase, as ketolase.
- 5